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Encounter with Unexpected Collagenase-1 Selective Inhibitor: Switchover of Inhibitor Binding Pocket Induced by Fluorine Atom

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Abstract—Phosphonamide-based inhibitors having trifluoromethyl moiety showed highly selective inhibition against MMP-1. A possible mechanism of the selectivity of MMP-1 inhibitors through the switchover of the binding pocket was speculated by computational calculations. As a consequence of the unexpected selectivity, the specific interaction of CF_3 group of the inhibitor and Arg214 in the S1′ pocket of MMP-1 conducted a low binding energy.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent metalloproteinases that have been shown to play a significant physiological role in extracellular matrix remodeling.[1] The implication of MMPs in a number of pathological processes has been reported, and, thus, they are considered to be important therapeutic targets for the treatment of a wide array of disease processes such as rheumatoid arthritis, tumor metastasis, multiple sclerosis, and congestive heart failure.[2] It has been reported that side effects were observed in the clinical studies of MMP inhibitors, because they showed broad-spectrum inhibition.[3] Therefore, specific inhibitors of MMPs are considered to be attractive targets in drug discovery research. In general, the studies of selective inhibitors for MMPs have focused on the optimization of the S1′ binding groups of the inhibitors, because X-ray analyses of the enzyme–inhibitor complex suggested that the S1′ pocket is a selectivity pocket for MMP inhibitors.[4–6] However, such an optimization strategy seems to be limited in elucidating the selectivity, because of their structural homology. We describe herein the first example of fluorine atom-induced highly selective inhibitors for collagenase-1, a novel concept for the design of selective inhibitors for MMPs.

Collagenase-1 (MMP-1) is known to cleave fibrillar collagens, and has been implicated in the process of arthritis.[7] On the other hand, it has been thought that the inhibition of MMP-1 may cause a side effect, such as musculoskeletal syndrome.[8] Therefore, a specific inhibitor for MMP-1 would be a very useful tool for investigating the physiological role of MMP-1 in vivo.

Recently, we demonstrated that the phosphonamide derivatives exhibited potent inhibition against MMPs.[9] Only the (*R*)-isomer **1a** at the phosphorus atom was found to be a potent inhibitor of MMP-1, -3 and -9, while the (*S*)-isomer **1b** was inactive for all MMPs (Table 1). This observation can be explained by the binding mode of the inhibitor in the active site of the MMPs. As shown in Figure 1a, the (*R*)-isomer could

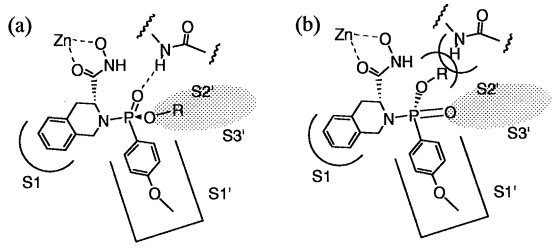

Figure 1. Expected binding mode of the phosphonamide inhibitors in MMPs.

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Table 1. In vitro profile of the phosphonamide derivatives

Compd	R	K_i (nM)[a]		
		MMP-1	MMP-3	MMP-9
1a (*R*)	CH_2CH_3	4.59	5.20	5.05
1b (*S*)	CH_2CH_3	>850	>650	>800
2a (*R*)	CH_2CH_2F	10.5	9.07	10.5
2b (*S*)	CH_2CH_2F	686	>650	>800
3a (*R*)	CH_2CHF_2	6.00	6.67	4.97
3b (*S*)	CH_2CHF_2	125	>650	483
4a (*R*)	CH_2CF_3	6.57	6.75	3.68
4b (*S*)	CH_2CF_3	35.6	617	358
5a (*R*)	$CH_2CH_2CF_3$	2.21	3.33	4.46
5b (*S*)	$CH_2CH_2CF_3$	6.23	>650	411
6a (*R*)	$CH_2CH_2CH_2CF_3$	10.5	29.4	5.53
6b (*S*)	$CH_2CH_2CH_2CF_3$	13.2	>650	>800

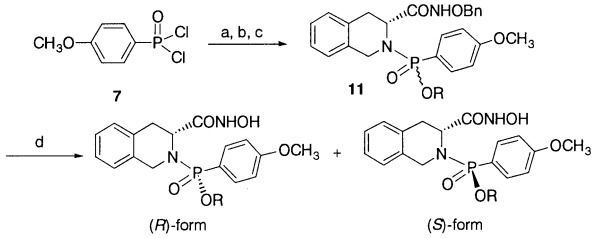
[a]See ref 9(a) for assay conditions.

bind to MMPs in the following fashion: the *p*-methoxyphenyl group attached to the phosphonamide would bind to the S1′ pocket of the MMPs, and one of the oxygen atoms of the phosphonamide would H-bond with the N–H of the mainchain of the MMPs. On the other hand, the (*S*)-isomer could not bind to the MMPs, because of the steric hindrance of the ester group attached to the phosphonamide (Fig. 1b).

In the course of the study, we have found that the introduction of fluorine atoms into the ester moiety of the (*S*)-isomers led to highly potent and selective inhibition against MMP-1. Therefore, we have investigated the effects of fluorine atoms on the activity and selectivity profile in more detail.

The synthesis of compounds **2**–**6** is shown in Scheme 1. Commercially available *p*-methoxyphenylphosphonic dichloride **7** was treated with the appropriate sodium alkoxide to provide the diester **8**, and then was selectively hydrolyzed by potassium hydroxide to give the corresponding monoester, which was converted to the corresponding phosphonyl chloride **9** by refluxing with thionyl chloride in the presence of catalytic DMF. The phosphonyl chloride **9** was then coupled with the (3*R*)-1,2,3,4-tetrahydroisoquinoline derivative **10** in THF in the presence of diisopropylethylamine (DIEA), to yield the (3*R*)-phosphonamide **11** as a mixture of diastereomers (1:1). Deprotection of the benzyl group in **11** with 10% Pd/C gave the diastereomerically pure two hydroxamic acids, which were successfully separated by HPLC purification.[10] The compound **1b** was crystallized and analyzed by X-ray diffraction analysis,[9b] and the stereochemistry at phosphorus atom of compound **1b** was determined to be *S* configuration. The stereochemistries of the other compounds were assigned by a characteristic signal pattern in ^{1}H NMR, especially in an aromatic region.

Compounds **2**–**6** were evaluated for the inhibition of MMP-1, -3 and -9 (Table 1).[11] Whereas the (*S*)-isomer of the ethyl ester derivative (**1b**) showed no inhibition in the nanomolar range against MMPs, the (*S*)-isomers of the fluorine-containing ester derivatives exhibited inhibitory activity against MMP-1. An increase in the number of fluorine atoms resulted in a dramatic



Scheme 1. (a) ROH, NaH, THF; (b) KOH, then $SOCl_2$, cat DMF; (c) (3*R*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinoline-3-hydroxamide (**10**), diisopropylethamine, THF; (d) H_2, Pd/C.

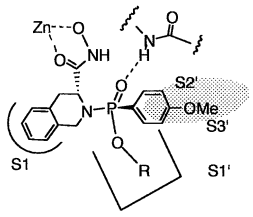


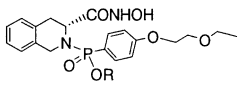
Figure 2. Proposed binding mode of (*S*)-inhibitors in MMP-1.

increase in the inhibitory activity for MMP-1, while little or no inhibition of MMP-3 and -9 was observed. The trifluoroethyl ester **4b** showed excellent inhibitory activity and selectivity against MMP-1 (K_i: 35.6 nM, selectivities for MMP-3 and -9: 17-fold and 10-fold, respectively). The extension of the methylene chain resulted in a further increase in the inhibitory activity for MMP-1, and it was found that the trifluoropropyl ester in **5b** was the optimum substituent. The K_i value of compound **5b** against MMP-1 was 6.23 nM, and the selectivities for MMP-3 and -9 were significantly increased to >104-fold and 66-fold, respectively.

This unexpected appearance of the inhibitory activity of the (*S*)-isomer could be explained by the switching of the binding mode in MMP-1, as shown in Figure 2. This new binding mode would bring the ester moiety in the (*S*)-isomer into the S1′ pocket, and therefore, the conflict of the ester moiety with the enzyme would be alleviated. To confirm this novel binding mode, we have synthesized compounds **12**–**13**. The introduction of long alkoxy chains to the *para*-position of the phenyl ring, such as the ethoxyethoxyphenyl group, is known to eliminate the inhibitory activity against MMP-1,[9] because of the small, closed shape of the S1′ pocket of MMP-1.[4] If the trifluoroalkylated (*S*)-isomer binds as in Figure 1b, then the (*S*)-isomers of the ethoxyethoxyphenyl derivatives could not inhibit MMP-1. Compounds **12**–**13** were synthesized from diethyl 4-(2-ethoxyethoxy)phenylphosphonate using a procedure to that described above.[12]

As expected, compound **13b** showed potent inhibitory activity against MMP-1, while the activity for MMP-1 of the (*R*)-isomer **13a** was completely lost (Table 2). Compound **13b** also showed a selective inhibition profile (K_i: >650 and >800 nM for MMP-3 and -9, respectively). As seen from Figure 2, the ethoxyethoxyphenyl group of (*S*)-isomers would be directed toward the S2′/S3′ site and have the potentiality to interact with these sites. Therefore, the weak activity for MMP-1 observed with

Table 2. In vitro profile of the phosphonamide derivatives



Compd	R	K_i (nM)[a]		
		MMP-1	MMP-3	MMP-9
12a (*R*)	CH_2CH_3	>850	30.0	14.2
12b (*S*)	CH_2CH_3	573	>650	>800
13a (*R*)	$CH_2CH_2CF_3$	>850	47.5	24.1
13b (*S*)	$CH_2CH_2CF_3$	31.4	>650	>800

[a]See ref 9(a) for assay conditions.

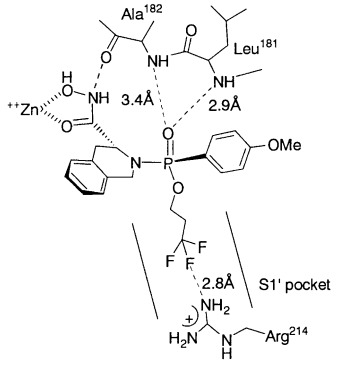


Figure 3. The interactions of compound **5b** bound to MMP-1.

compound **12b** would be induced by such a new interaction.

To explain the observed selectivity, the complex model of MMP-1 and compound **5b** was constructed (Fig. 3).[13] This model strongly suggested the possibility of an H-bond interaction between the fluorine atom and Arg 214 at the bottom of the S1′ pocket. One of the fluorine atoms in the trifluoromethyl group was placed 2.8 Å away from the terminal nitrogen atom of Arg 214. As this Arg 214 is known to undergo a conformational change to accommodate inhibitors with large substituents,[4] MMP-1 could tolerate the chain length of the ester group (**4b**, **5b**, and **6b**). It has been reported that the specific interaction of a CF_3 group and an Arg residue could induce a conformational change within an enzyme.[14] On the other hand, the shape of the S1′ pockets of MMP-3[15] and -9[16] is large and deep, which is much different from that of MMP-1. Therefore, the fluorine atom of the (*S*)-form inhibitors could not interact as in MMP-1, and thus the change of the binding mode would be difficult to be induced. However, it seems not only the H-bond interaction between the fluorine atoms and Arg 214 but the hydrophobic interaction between the fluorinated ester and the hydrophobic S1′ pocket slightly contributed to the binding affinity. So the fluorinated compounds **3b**, **4b** and **5b** would show weak activity against MMP-9 without Arg 214 equivalent in the S1′ pocket. However, a more detailed study, such as an X-ray analysis of the enzyme–inhibitor complexes, will be required to understand the observed results. These studies are now in progress.

In summary, we have described the first example of a highly selective MMP inhibitor by the fluorine atom-induced switching of the binding mode. The (*S*)-form of the 3,3,3-trifluoropropyl ester derivative (**5b**) showed potent inhibitory activity against MMP-1 with a highly selective profile. The different binding mode of this type of compound is likely to enable the inhibition of MMP-1. This study reveals the potential of the phosphonamide derivatives as a new type of MMP inhibitor, and provides a novel concept for the design of selective inhibitors.

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References and Notes

1. Woessner, J. F., Jr. *FASEB J.* **1991**, *5*, 2145.
2. Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735 a review and references therein.
3. (a) Nemunaitis, J.; Poole, C.; Primrose, J.; Rosemurgy, A.; Malfetano, J.; Brown, P.; Berrington, A.; Cornish, A.; Lynch, K.; Rasmussen, H.; Kerr, D.; Cox, D.; Millar, A. *Clin. Cancer Res.* **1998**, *4*, 1101. (b) Heath, E. I.; Grochow, L. B. *Drugs* **2000**, *59*, 1043.
4. Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campbell, J. A.; Walker, K. A. M.; Martin, R.; Wart, H. V.; Browner, M. F. *Nat. Struct. Biol.* **1999**, *6*, 217.
5. Pavlovsky, A. G.; Williams, M. G.; Ye, Q.-Z.; Ortwine, D. F.; Purchase, C. F., II; White, A. D.; Dhanaraj, V.; Roth, B. D.; Johnson, L. L.; Hupe, D.; Humblet, C.; Blundell, T. L. *Protein Sci.* **1999**, *8*, 1455.
6. Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
7. (a) Mitchell, P. G.; Magna, H. A.; Reeves, L. M.; Lopresti-Morrow, L. L.; Yocum, S. A.; Rosner, P. J.; Geoghegan, K. F.; Hambor, J. E. *J. Clin. Invest.* **1996**, *97*, 761. (b) Billinghurst, R. C.; Dahlberg, L.; Ionescu, M.; Reiner, A.; Bourne, R.; Rorabeck, C.; Mitchell, P.; Hambor, J.; Diekmann, O.; Tschesche, H.; Chen, J.; Wart, H. V.; Poole, A. R. *J. Clin. Invest.* **1997**, *99*, 1534.
8. Leff, R. L. *Ann. N.Y. Acad. Sci.* **1999**, *878*, 201.
9. (a) Sawa, M.; Kiyoi, T.; Kurokawa, K.; Kumihara, H.; Yamamoto, M.; Miyasaka, T.; Ito, Y.; Hirayama, R.; Inoue, T.; Kirii, Y.; Nishiwaki, E.; Ohmoto, H.; Maeda, Y.; Ishibushi, E.; Inoue, Y.; Yoshino, K.; Kondo, H. Submitted for publication. (b) Kurokawa, K.; Kanehisa, N.; Sawa, M.; Kondo, H.; Kai, Y. *Acta Cryst.* **2001**, *E57*, 906.
10. Satisfactory characteristics data were obtained for all new compounds. Characteristics are given for a representative compound: **5b**; colorless solids; ^{1}H NMR (DMSO-d_6) δ 2.70–2.85 (m, 2H), 2.95–3.15 (m, 2H), 3.75 (s, 3H), 3.92 (dd, J=7.5 and 16.1 Hz, 1H), 4.20–4.50 (m, 3H), 4.55–4.60 (m, 1H), 6.90–7.00 (m, 3H), 7.00–7.15 (m, 3H), 7.53 (dd, J=8.8 and 12.5 Hz, 2H), 8.78 (s, 1H), 10.59 (br s, 1H); MALDI-TOF MS 497 $[M+K]^+$, 481 $[M+Na]^+$, 459 $[M+H]^+$. Anal. calcd for $C_{20}H_{22}F_3N_2O_5P$: C, 52.41; H, 4.84; N, 6.11. Found: C, 52.15; H, 4.94; N, 6.02.
11. Recombinant human collagenase-1 (MMP-1), stromelysin 1 (MMP-3), and gelatinase B (MMP-9) were used in our studies [see ref 9(a) for assay conditions].

12. Satisfactory characteristics data were obtained for all new compounds. Characteristics are given for a representative compound: **13b**; colorless solids; ^{1}H NMR (DMSO-d_6) δ 1.08 (t, J = 7.0 Hz, 3H), 2.65–2.85 (m, 2H), 2.95–3.15 (m, 2H), 3.45 (q, J = 7.0 Hz, 2H), 3.60–3.70 (m, 2H), 3.92 (dd, J = 7.5 and 16.5 Hz, 1H), 4.05–4.10 (m, 2H), 4.20–4.45 (m, 3H), 4.50–4.60 (m, 1H), 6.90–7.00 (m, 3H), 7.00–7.15 (m, 3H), 7.52 (dd, J = 8.7 and 12.5 Hz, 2H), 8.77 (d, J = 1.5 Hz, 1H), 10.59 (d, J = 1.5 Hz, 1H); MALDI-TOF MS 555 $[M+K]^+$, 539 $[M+Na]^+$, 517 $[M+H]^+$. Anal. calcd for $C_{23}H_{28}F_3N_2O_6P$: C, 53.49; H, 5.46; N, 5.42. Found: C, 53.29; H, 5.53; N, 5.37.

13. Models of MMP-1 complexed with the phosphonamide inhibitors were constructed based on the crystal structure of a MMP-1/peptide-based inhibitor complex (PDB code, 2TCL[5]). Firstly, the phosphonamide inhibitor was placed in the active site of the MMP-1 to occupy the S1′ pocket with the R group. Then, the hydroxamate unit of the phosphonamide inhibitor was superimposed on that of the peptide-based inhibitor. The peptide-based inhibitor was replaced by the phosphonamide inhibitor, and the obtained protein–ligand complex was subsequently energy minimized treating all ligand atoms and retaining all protein atoms. Docking and energy minimizations were performed with the TRIPOS force field within the SYBYL program. All computations were performed on an Octane Silicon Graphics workstation. The two complexes (**1b**/MMP-1 and **5b**/MMP-1) were energy minimized and the differences of the binding energies between **1b**/MMP-1 and **5b**/MMP-1 were obtained. As a result, the total binding energy of **5b**/MMP-1 was lower than that of **1b**/MMP-1, and the energy difference is about 7 kcal/mol. This difference is equal to 2.5×10^5 times difference of K_i value. Moreover, it was found that the interactions of the CF_3 and Arg214 mainly contributed to this stabilization.

14. Mattos, C.; Giammona, D. A.; Petsko, G. A.; Ringe, D. *Biochemistry* **1995**, *34*, 3193.

15. (a) Becker, J. W.; Marcy, A. I.; Rokosz, L. L.; Axel, M. G.; Burbaum, J. J.; Fitzgerald, P. M. D.; Cameron, P. M.; Esser, C. K.; Hagmann, W. K.; Hermes, J. D.; Springer, J. P. *Protein Sci.* **1995**, *4*, 1966. (b) Esser, C. K.; Bugianesi, R. L.; Caldwell, C. G.; Chapman, K. T.; Durette, P. L.; Girotra, N. N.; Kopka, I. E.; Lanza, T. J.; Levorse, D. A.; MacCoss, M.; Owens, K. A.; Ponpipom, M. M.; Simeone, J. P.; Harrison, R. K.; Niedzwiecki, L.; Becker, J. W.; Marcy, A. I.; Axel, M. G.; Christen, A. J.; McDonnell, J.; Moore, V. L.; Olszewski, J. M.; Saphos, C.; Visco, D. M.; Shen, F.; Colletti, A.; Krieter, P. A.; Hagmann, W. K. *J. Med. Chem.* **1997**, *40*, 1026.

16. Olson, M. W.; Bernardo, M. M.; Pietila, M.; Gervasi, D. C.; Toth, M.; Kotra, L. P.; Massova, I.; Mobashery, S.; Fridman, R. *J. Biol. Chem.* **2000**, *75*, 2661.